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TITLE:
"Intermittent hypoxia elicits prolonged restoration of motor function in human SCI"

PRINCIPAL INVESTIGATOR:
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14. ABSTRACT

At the University of Wisconsin, progress was made in the second year of this award, although our ability to complete the project was limited by the departure of a key person. Thus, we applied for a no-cost-extension and will be completing the work within this new, three year time frame. The fundamental goal in Wisconsin is to test the hypothesis that repetitive intermittent hypoxia combined with treadmill training significantly increases protein expression of proteins associated with spinal motor plasticity (BDNF and its high affinity receptor, TrkB). These assessments will complement behavioral data collected at the University of Saskatchewan, and parallel experiments in humans with SCI at Emory University and the Rehabilitation Institute of Chicago. In this second year of the grant, tissues were processed for immunohistochemistry and the extensive densitometry analyis was pursued. Analyses are based on five treatment groups of rats with cervical injuries: 1) shelf controls; 2) sham surgery; 3) daily treadmill training for five days; 4) intermittent hypoxia for five days; and 5) combined intermittent hypoxia and treadmill training. Groups were collected at six time points, to determine the duration of changes in protein expression. In the next year, we plan to complete immunohistochemical analyses, combine our results with parallel behavioral studies at the two collaborating sites and prepare a manuscript for publication.

15. SUBJECT TERMS

Spinal Injury, Treatment, Intermittent hypoxia, humans, rats, BDNF

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Annual progress report:
Award Number W81XWH-10-1-0830
"Intermittent hypoxia elicits prolonged restoration of motor function in human SCI"
Research Completed at University of Wisconsin, Madison

Introduction
The fundamental goal of the experiments performed in this component of the translational partnership award is to assess changes in ventral spinal protein expression in rats with cervical spinal injuries following exposure to intermittent hypoxia alone, locomotor training alone, or combined intermittent hypoxia with locomotor training. We will then correlate these assessments with behavioral data from rats collected in our collaborators laboratory in Canada. Both experiments will then be correlated with observations made in humans with SCI following similar experimental interventions (data being collected in Atlanta and Chicago).

Body
Our strategy in this first year of this award was modified slightly to collect all rat tissues at the same time. The laborious protein assessments are being made in subsequent years of the award. At this point, we have collected all necessary tissues and completed approximately half of the immunohistochemistry analysis. Analyses via densitometry requires considerable time.

In specific, we collected tissues from the following 5 groups of rats with cervical spinal injuries:
- Sedentary rats exposed only to normal oxygen conditions
- Rats that received normal oxygen only, but with treadmill training (5 days)
- Rats that were sedentary, but were exposed to 5 successive days of acute intermittent hypoxia
- Rats that received combined intermittent hypoxia and treadmill training (5 days)
- Sham surgery rats that had no other experimental interventions.

In each of these experimental treatments, 3 rats were harvested at each of 6 time points (relative to treatments):
- 8 weeks post SCI with no other treatment (baseline)
- 9 weeks post-SCI; intermittent hypoxia (1 min of 10.5% O2 with 1 min normoxic intervals, 15 episodes) and/or treadmill training commenced at 8 weeks and 2 days; treatments were for 5 consecutive days; rats were sacrificed 1 hour after the final treatment.
- 10 weeks post-SCI or 1 week after treatments had ended
- 11 weeks post-SCI or 2 weeks after treatments had ended
- 13 weeks post-SCI or 4 weeks after treatments had ended
- 17 weeks post-SCI or 8 weeks after treatments had ended

Rats were anesthetized, perfused with paraformaldahyde, and the spinal cords were dissected. Tissues between cervical segment C7 and thoracic segment T1 were then sectioned with a microtome (40um sections). We also sectioned the site of injury (C2) and stained the tissues with Cresyl violet to document the injury. Currently, we are staining the C7 to T1 tissues for BDNF, TrkB and phosphorylated TrkB as described in our grant application. Overall, we successfully collected 88 tissues from 90 rats (2 rat tissues were not successfully perfused). Data will be available only after we complete immunohistochemistry for the specified proteins, and then complete densitometric analyses. This is a laborious process and will take a considerable portion of the next year.

Key Research Accomplishments (related to Statement of Work)
Established collaborative effort with routine communication between the three sites. In addition to email contact and phone calls, we met face to face three times during the year (once in Atlanta, once in New Orleans (SFN meeting) and once in Madison).

Research tasks completed at the Madison site are listed below in connection with their description in the Statement of Work.

Specific Aim 1, Task 1, Milestone #1: Obtain Animal and Human Use Approvals--Milestone accomplished

Specific Aim 1, Task 2

Subtask 2a: Perform spinal injuries and AIH treatment----task completed.
Subtask 2b: Quantify the expression of key proteins post-AIH---task underway
Subtask 2c: Correlate the expression of key proteins with limb functional recovery as determined in subtasks 1c and 1e----pending, awaiting completion of Task 1 and Subtask 2b.

Specific Aim 2, Task 6
Subtask 6a: Perform spinal injuries and AIH +/- locomotor training in the first cluster of naive rats prior to spinal injuries---task completed.
Subtask 6b: Quantify the expression of key proteins post-treatments in the first clister of rats---task underway
Subtask 6c: Correlate the expression of key proteins with limb functional recovery as determined in Subtasks 5b and 5d---pending, awaiting completion of Task 5 and subtask 6b.

Reportable Outcomes None, pending completion of our studies.

Conclusions We have made good progress in accordance with our experimental plan, although we were limited in our pace of immunohistochemical analysis due to loss of key personnel. Simultaneous analysis of protein expression in all groups will greatly enhance our ability to compare across groups using the semi-quantitative immunohistochemical methods proposed. Thus, our major goals in the coming year remain to complete the laborious analyses of protein expression, and then to correlate these assessments with behavioral data expected to be completed at the University of Saskatoon in Saskatchewan, Canada.

References None

Appendices None